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RECENT DEVELOPMENTS FOR *STAPHYLOCOCCUS AUREUS* VACCINES: CLINICAL AND BASIC SCIENCE CHALLENGES

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Abstract

Bacterial vaccines have made dramatic impacts upon morbidity and mortality caused by a number of common pathogens, but a vaccine to prevent *Staphylococcus aureus* infections has proven to be illusive. With successful bacterial vaccines, the organisms are all part of the transient flora, whereas, *S. aureus* is part of the normal human flora. This means that *S. aureus* has had a prolonged time to adapt to the host milieu and its defences. The failure of several staphylococcal antigens to protect humans from infection in vaccine clinical trials using active or passive immunisation has stimulated a re-examination of the fundamental assumptions about staphylococcal immunity in humans *vs*. animals, especially rodents. This has spurred an active debate about the appropriate models for vaccine development and an examination of our current understanding of the protective immunity in humans. A major factor in the development of previous bacterial vaccines was a biomarker that predicted human protection, *e.g.,* antibodies to tetanus toxoid or to pneumococcal polysaccharide. While antibodies against a number of staphylococcal antigens have proven to be an excellent biomarker for protection in rodents, these have not been translated to human infections. Thus, while much work remains, there is a growing consensus that T-cell immunity plays an important role in protecting humans. Moreover, the presence of anti-staphylococcal toxin antibodies correlates with reduced disease severity in humans. The most important recent advances concerning potential biomarkers, and the role of pre-existing immune status of vaccines in vaccine-associated mortality are considered in this review.

Keywords: *Staphylococcus aureus*, immunity, Th17, clinical trials, vaccine.

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Introduction

The epidemiology of *S. aureus* establishes a need for a vaccine. *S. aureus* is the number one cause for children requiring hospitalisation and surgery, and the leading cause of bacteraemia in people > 65 years old (Proctor 2012a; Proctor 2012b). Another recent review (Tong *et al.,* 2015) notes the impressive rates of *S. aureus* infections /100,000 population/y. Over that past decade bacteraemia rates = 20-38, which jump to > 100 for people over age 70; 1,960 for HIV positive adults, and 66.5 for Blacks (as compared to Whites = 27.7) When considering either MSSA or MRSA, there is a 25 % 12-week mortality in cardiac device infections and a 7-21 % mortality with intravascular catheter blood stream infections (Tong *et al.*, 2015). The rates of invasive *S. aureus* infections are comparable to the pre-vaccine rates for *Haemophilus influenzae* (David and Daum, 2010; Millar *et al.*, 2005). With skin and soft tissue infections, the rates for patients coming from community or basic training recruits range from 0.35/1000 to 4/1000 *per* year (Liu *et al*., 2008; Morrison-Rodriguez *et al.*, 2010). A large multi-city study in the USA established a mortality rate of 18,650 MRSA deaths/year in the USA (Klevens *et al.*, 2007). Finally, staphylococci are the most frequent pathogen involved in joint infections (Fisher *et al.*, 2015; Kapadia *et al.,* 2015; Lin *et al.*, 2015; Parvizi *et al.* 2015). Therefore, there is clearly a need for a vaccine to protect against staphylococcal infections.

When considering vaccines, there are different levels of protective efficacy. The highest level would be prevention of infection. The next level would be reduced mortality. The lowest barrier would be reduced severity. Clearly, the last two levels are often directly related. A particular challenge comes with implant infections as the presence of biomaterials dramatically reduces the inoculum needed to establish an infection. This was first demonstrated in humans when Elek showed that the inoculum was reduced by at least 10^5 fold when a suture was placed in the wound as compared to subcutaneous inoculation into skin (Elek and Conen, 1957). The presence of a foreign body also increases the difficulty in treating with antibiotics as biofilms of organisms show resistance even when the bacteria are susceptible in the microbiology laboratory (reviewed in McCarthy *et al.*, 2015).

When considering bacterial vaccines, all successful vaccines have been developed when a biomarker for protective immunity was established. For example, anti-capsular antibody against *Streptococcus pneumoniae* and anti-toxin antibody against tetanus toxoid correlated directly with protection against human *S. pneumoniae* and

Clostridium tetani. Much of the difficulty in developing a preventive *S. aureus* vaccine stems from the fact that biomarker(s) for human protective immunity are unknown (Fowler and Proctor, 2014). In contrast, we do know that anti-toxin antibodies have been associated with reduced severity of human infections (Fowler and Proctor 2014; Fritz *et al.*, 2013; Rasigade *et al.*, 2011; Spalding *et al.*, 2012). With 90 % effectiveness for anti-toxin antibodies, one would anticipate that differences between controls and immunised to be achieved with 300 subjects in each group (Aman and Adhikari, 2014). Finally, another challenge for producing a *S. aureus* vaccine has been the fact that *S. aureus* produce many diseases and the strains of bacteria producing those diseases are dissimilar in terms of virulence factors (Laabei *et al.*, 2015; Lowy, 1998). Hence, one could reasonably ask which *S. aureus* disease does one want to address rather than trying to address all infections? As the goal of this review is to consider the role for vaccines in implant infections, it will focus upon the prevention of disease, and the challenges for achieving this goal.

Completed clinical trials

What have we learned from the many clinical trials of various vaccine candidate antigens? To date, all of the clinical vaccine trials have been aimed at preventing or treating *S. aureus* diseases (Proctor 2012a; Proctor 2012b). Detailed reviews of the clinical trials have recently been reviewed and specifics will not be repeated in this article (Fowler and Proctor, 2014). Both passive and active immunisation has been tried (Fowler and Proctor, 2014; Jansen *et al.*, 2013). The clinical trials have been based upon protective efficacy in animals, usually mice, and the immune marker has been opsonophagocytic antibody (Fowler and Proctor, 2014; Jansen *et al.,* 2013; Proctor, 2012a; Proctor, 2012b). All of the active immunisations showed increases in opsonophagocytic antibodies in human volunteers, as well as in subjects in the clinical trials. Nevertheless, all clinical trials have failed, despite strong protective responses in animals (Fowler and Proctor, 2014; Proctor 2012a; Proctor 2012b).

Developing a vaccine that prevents implant infections has been a goal for vaccine production (Montanaro, 2011). This might be accomplished by reducing adhesion and/or biofilm formation to the foreign body, and this has been tested against a small number of antigens within some of the clinical vaccine trials wherein anti-ClfA, anti-SdrG, anti-capsular polysaccharide (types 5 and 8) were tested using either active or passive immunisation (DeJonge *et al.*, 2007; Rupp *et al.*, 2007; Shinefield *et al.*. 2002; Weems *et al.*, 2006). None of these antigens have proven successful in human vaccine trials. However, an anti-alpha toxin antibody (Brady *et al.*, 2013), anti-glucosaminidase antibody (Varrone *et al.*, 2014) and accumulation protein antibody (Yan *et al.,* 2014) have shown efficacy in murine models. Perhaps one of these new antigens will prove successful. While complete prevention is a very high hurdle, the strategy to combine antibodies with antibiotics may be efficacious as the antibodies will reduce biofilm and clumping of bacteria and thereby enhance antibiotic activity.

On-going clinical human vaccine trials

Some comments can be made about the five vaccine trials and the human host response to invasive *S. aureus* infections where data are still pending (Table 1).

The Pfizer trial is examining protection from infections in patients undergoing lumbar spinal fusion. Except for

Table 1. Information from the clinical trials.gov web site (https://clinicaltrials.gov/ct2/results?term=staphylococcus&Search=Search) searched for the term "staphylococcus" and from company web sites, e.g., GSK Pipeline report (see Web References). All of the trials have demonstrated the production of antibodies, and no safety concerns in the Phase I and II trials (where applicable) as reported by data at the web sites

Company	Antigens	Phase	Comments
Pfizer	ClfA, MntC, CP 5, CP8	II (Lumbar surgery)	3 of 4 Ags already tested before
NovaDigm	rAls3p-N (NDV-3) for Ab and T cell responses	IIb	Single, novel antigen for *S. aureus* and *C. albicans* RVVC
NABI/GSK	rLukS-PV, α-toxin	I	Secreted proteins; no longer listed in March 2015 pipeline report
GSK	Tetravalent	I	Unknown antigens; no longer listed in March 2015 pipeline report
Novartis	4 proteins	I	Based upon opsonophagocytic Ab; no data are available
Vaccine Research Intl	Whole cell (SA75)	I	Measured Ab against CAN, ClfA, FnBP, Eap; no data provided on thefunction of the Abs

Ab = antibody; Ag = antigen; rAlsp-N = recombinant N terminus of *C. albicans* protein; CAN = collagen binding protein; ClfA = clumping factor; CP5 and CP8 = capsular polysaccharide types 5 and 8; Eap = extracellular adhesion protein; MntC = manganese transport protein C; rLukS-PV = a recombinant PVL = Panton-Valentine leukocidin; RVVC = recurrent vulvovaginal candidiasis; Th17 = T helper 17 cell; TLR = toll-like receptor. These are surface antigens on *S. aureus* that are being developed as vaccine components.

MntC, the other three antigens (Nissen *et al.*, 2015) have been tested in other vaccine trials and failed. There are no study results posted on the web sites. However, a follow-up Phase III trial was just published on the types 5 and 8 capsular antigens as vaccine candidates wherein a second dose was added at 35 weeks for patients on haemodialysis, and this trial failed to show protection as well (Fattom *et al., 2015*). The NDV-3 trial sponsored by NovaDigm is using a novel recombinant protein from *C. albicans* that was found to provide cross protection against *S. aureus* infections that was mediated by Th17 cells in mice (Schmidt *et al.,* 2012; Spellberg *et al.*, 2008). The Phase I trial is followed up with a Phase II trial aimed at preventing recurrent vulvovaginal candidiasis. One assumes that clinical trials for *S. aureus* will be forthcoming, but none are yet scheduled. Two trials involving GSK antigens (rLukS-PV/α-toxin and a Tetravalent vaccine) have finished phase I, but the March 2015 pipeline report no longer has these trials listed as on going. Only the safety data from phase I have been released. A Novartis clinical trial using four proteins, which have not been specified, may also be on-going, but no data beyond the phase I trial are available. The basis for the Novartis trial is opsonophagocytic activity, whose limitations have been discussed above. Vaccine Research International is studying a whole cell vaccine, based upon *S. aureus* 75, and the data available report on antibodies directed against staphylococcal adhesins. There are no data provided about the functional activity of these antibodies, but the goals are prevention of infection; therefore, one assumes that the function would be prevention of colonisation. Finally, a NIAID-sponsored trial conducted through the University of Maryland started in 2009 and was completed in Sept 2014. The goals of this trial were to examine white blood cell responses using microarrays, Th17 activity, and TLRs responses. While this trial should provide human immune response data in invasive *S. aureus* infections, these much-needed results have not yet been published.

Conclusions from clinical trials

Several conclusions may be drawn explaining the poor outcomes of the clinical trials. First, the protective immune response (opsonophagocytic antibodies) found in the animal models used to date have not predicted efficacy in humans. Second, excess mortality was seen in the V710 (anti-IsdB antigen) vaccinated and *S. aureus* infected group, which was associated with systemic inflammatory response and multi-organ failure (Fowler *et al.*, 2013). A similar disturbing finding occurred with anti-type 5 and 8 pooled human anti-capsular antibodies (Altastaph), wherein there was a trend toward higher mortality in the vaccinated group (23 % *vs.* 11 %, $p = 0.42$) as well as more adverse events (95 % in Altastaph group) (Rupp *et al.*, 2007). Third, anti-*S. aureus* antibodies are not a biomarker for protection against human *S. aureus* infections (Fowler and Proctor, 2014; Montgomery *et al.*, 2014; Salgado-Pabón and Schlievert, 2014; Verkaik *et al.*, 2010). Fourth, *S. aureus* causes a very wide variety of diseases, and it may be asking too much for a single vaccine to prevent all of them as different strains of *S. aureus* have differing propensity for causing certain types of infections. Finally, *S. aureus* is a part of the normal flora and has evolved many mechanisms for thwarting the human immune response, especially opsonophagocytic processes (Bestebroer *et al.*, 2010; Lu *et al.,* 2014; Pauli *et al.*, 2014; Serruto *et al.,* 2010; Spaan *et al.*, 2013; van Kessel *et al.,* 2014). In these reviews, we see that *S. aureus* has essentially every step of the immune response blocked by its vast array of proteins. Examples include, inhibition of neutrophil attachment to endothelial cells by SSL-5, SSL-6 and Eap; inhibition of neutrophil interaction with chemoattractants by SSL-10, CHIPs, and FLIPr; blocking of antibody interactions with neutrophils by SSL-7, SSL-8, and protein A; and inhibition of complement by Ecb, SCIN, and CHIPS. A more complex vaccine approach might include neutralising several of the staphylococcal factors that inhibit the immune system as well as adding vaccine antigens.

In retrospect, the failure of opsonophagocytic antibodies to protect humans is not surprising. While opsonophagocytic antibodies are a clear biomarker for *H. influenzae* and *S. pneumoniae*, patients with agammaglobulinaemia (genetic or acquired complete lack of immunoglobulins) show no increase in the incidence of *S. aureus* infection (reviewed in Fowler and Proctor, 2014). Of course, neutralising antibodies to staphylococcal toxins have correlated with reduction in the severity of *S. aureus* infections (Fowler and Proctor, 2014; Salgado-Pabón and Schlievert, 2014), but these antibodies are less likely to prevent infections. Moreover, the use of rodent models for predicting human responses to staphylococcal infections for either protective efficacy (Proctor 2012a; Proctor 2012b; Salgado-Pabón and Schlievert, 2014) or human inflammatory responses to sepsis (Seok *et al.*, 2013), have been poor.

A very recent review by (Pozzi *et al*., 2015) attempts to address the role of antibody and animal models in the design of staphylococcal vaccines. Unfortunately, this review contains major errors because of a lack of understanding of clinical medicine and basic epidemiology. In particular, there are major problems with the associations drawn between hypogammaglobulinaemia and the increased incidence of *S. aureus* infections. For example the authors fail to separate the occurrence of *S. aureus* pneumonia in some patients with XLA and an increased incidence. The rate of *Staphylococcus sp.* was not increased above background. Moreover, patients with disorders in their skin (atopic dermatitis, juvenile pityriasis rubra, *etc*.) have a failure of the barrier function of the skin; therefore, they have more *S. aureus* infections. This happens in all people with loss of barrier function and hypogammaglobulinaemia again is not the cause of the more frequent infections. Heavy colonisation with *S. aureus* is a risk factor for invasive *S. aureus* infections so skin barriers are important. In another paper cited in the (Pozzi *et al*., 2015) review, an IgG infusion used by (Castanet *et al*., 1994) was claimed to cause improvement of *S. aureus* folliculitis. This is a single anecdotal case. More importantly, this case report has multiple shortcomings. First, there is no description of the organism. Is it a heavy toxin producer? Production of leukocidins and haemolysin has been associated with more severe skin infections. Clearly, this and previous reviews

have emphasised that neutralisation of toxins can reduce the severity of *S. aureus* disease (Proctor, 2012a; Proctor 2012b, Fowler and Proctor, 2014). However, anti-toxins have not yet been shown to prevent *S. aureus* infections. Second, as noted in the Castanet case report, people with these skin diseases have more viral and bacterial infections due to loss of barrier function of the skin. There were no viral cultures taken in this patient; therefore, the IVIG may have treated a concurrent viral infection, which then allowed clearing of the *S. aureus* infection. Third and most importantly, there was no testing of the patient's neutrophils in the presence and absence of the IVIG against the *S. aureus* isolated from the patient. Hence, the assertion that hypogammaglobulinaemia played a role in clearing the infection is simply speculation. Fourth, T-cell dysfunction has been reported with juvenile pityriasis rubra pilaris (Shvili *et al.*, 1987), which might account for the cutaneous *S. aureus* infections. (Castanet *et al*., 1994) examined the numbers of T-cell subsets, but they did not test the function of the T cells. Fifth, the IVIG may have altered the skin disease thereby improving the barrier function, and it is noteworthy that no biopsy of the skin is reported after the IVIG therapy. In support of this idea is the fact that it took over 2 months for the IVIG to bring improvement to the *S. aureus* skin infections. If the IVIG were acting to improve opsonic activity in the patient, then the response should have been almost immediate (certainly within a week) rather than after two months. Thus, this single case was poorly studied and complicated because the patient continued to receive antibiotics; therefore, the impact of IgG cannot be linked directly to the clearance of *S. aureus*. Other statements are made about IgG infusions improving *S. aureus* infections, but these are non-referenced in the (Pozzi *et al*., 2015) review. Further attempts are made to implicate IgG, for example, patients with chronic lymphocytic leukaemia (CLL) are mentioned, but these patients are often treated with agents that cause neutropaenia and with prednisone, which suppresses T-cell immunity. In addition, in the later stages of CLL there is often a decrease in cell-mediated immunity. The CLL data are not at all convincing that hypogammaglobulinaemia had anything to do with their *S. aureus* infections. Finally, "rheumatologic disorders" are thrown-in to support of hypogammaglobulinaemia as a cause of *S. aureus* infections, but there are clearly other immunological reasons for *S. aureus* infections in these patients, such as rheumatoid factor, that blocks neutrophil function; treatment with prednisone that depresses cell-mediated immunity, and neutropaenia is frequent in a number of "rheumatologic disorders". In summary, the information in the (Pozzi *et al.,* 2015) review is not at all convincing that hypogammaglobulinaemia plays any role in increasing the incidence of *S. aureus* infectious diseases.

Other arguments are made by (Pozzi *et al*., 2015) for the value of antibodies based upon animal models, but we have ample clinical data from the failed human clinical vaccine trials that these models do not predict success in humans. Another argument for antibodies being important is that people develop antibodies to staphylococcal antigens after *S. aureus* infections and after colonisation shortly following birth. This only shows that the patients had an immune response, which would involve T-cell immunity, but it does not establish antibodies are being protective. Finally, data for the protective effect of anti-toxin antibodies are provided, and these comments are in agreement with this and previous reviews as being valuable.

The (Pozzi *et al*., 2015) review continues with a very large amount of elegant murine immunology, which has not been shown to be relevant to human immune response against *S. aureus*. Demonstration that phagocyte subsets and lymphocyte clonal deletion are key elements in the failure of the staphylococcal vaccines would be a major step in developing a preventive *S. aureus* vaccine, but this will require considerable work to show that it has occurred in the failed human clinical vaccine trials or in a prospective clinical trial.

The lack of biomarkers and predictive animal models strongly makes the case for having more research into the human immune response before going ahead with more vaccine trials. What do we know about the human protective immune response against *S. aureus*?

Human adaptive immunity against *S. aureus*

A model showing the immune cells and cytokines involved in human adaptive immunity to *S. aureus* is provided in Fig. 1. This model does not show the plethora of staphylococcal extracellular factors used to thwart the actions of antibodies, complement, and phagocytes because these have been extensively recently reviewed (Bestebroer *et al.*, 2010; Spaan *et al.*, 2013; Serruto *et al.*, 2010; van Kessel *et al.*, 2014).

People with defects in cellular immunity have an increased incidence of *S. aureus* infections (Crum-Cianfione *et al.,* 2009; Ishigame *et al.*, 2009). Similarly, people with neutropaenia or defects in neutrophil function develop more *S. aureus* infections than normal people (Donabedian and Gallin, 1983; Ma *et al.*, 2008; Minegishi *et al.*, 2009; Quilty *et al.*, 2009; White and Gallin, 1986) as neutrophils play a major role in killing invasive *S. aureus* (Kobayashi *et al.*, 2010). Where do cellular immunity and neutrophil problems coincide? This occurs in people who have defects in Th17 cells because the Th17/IL-17 arm of the immune system is used to call in and to activate neutrophils at sites of *S. aureus* invasion (Ishigame *et al.*, 2009; Ma *et al.*, 2008; Minegishi *et al.*, 2009). Moreover, Th17 activation also primes mucosal and skin surfaces to produce cationic antimicrobial peptides, which enhances the protective barrier against *S. aureus* invasion (Minegishi *et al.*, 2009). Cytokines IL-6 activates Th17 while IL-10 reduces Th17 activation (Maródi *et al.,* 2012; Puel *et al.*, 2008). Phenol soluble modulins (PSMs) produced by *S. aureus* stimulate macrophages to produce more IL-10 (Schreiner *et al.*, 2013), thereby down-regulating Th17 activation. Blockage of IL-6 with autoantibodies and increased levels of IL-10 are associated with more *S. aureus* infections in humans (Maródi *et al.,* 2012; Puel *et al.*, 2008). Very recently, IL-26, which is a downstream cytokine in the Th17 pathway, not only functions as a cytokine, but it also has antimicrobial peptide activity (Braum *et al.*, 2012; Meller *et al.*, 2015). Also recently,

bone marrow dendritic cells and macrophages, which normally kill *S. aureus*, were found to be a safe haven for *S. aureus* strains that displayed high activity of the Agr quorum-sensing system (O'Keeffe *et al.,* 2015). Finally, the staphyloccidal activity of dendritic cells is enhanced by vitamin D and patients that are vitamin D deficient are more likely to have more nasal carriage, skin infections, and more invasive infections (Olsen *et al.*, 2012; Thomason *et al.,* 2015; van der Does *et al.*, 2014; Wang *et al.,* 2015).

Some of the antibodies tested in clinical trials have aimed at staphylococcal adhesins (Fowler and Proctor, 2014; DeJonge *et al.*, 2007; Weems *et al.*, 2006). The antibodies must bind these ligands and prevent attachment to host tissues. While higher affinity monoclonal antibodies have been produced, they have failed in clinical trials in terms of preventing or treating infections (Bebbington and Yarranton, 2008). The affinity of *S. aureus* adhesins directly correlates with the development of invasive infection (Casillas-Ituarte *et al.*, 2012; Lower *et al.*, 2011); hence, this may have clinical relevance. One factor that may contribute to the failure of monoclonal antibodies to prevent infections is that *S. aureus* surface proteins have multiple binding sites with very high dissociation constants (Provenza *et al.,* 2010; Ross *et al.,* 2012); therefore, even relatively high affinity MAbs may not be able to compete with the staphylococcal adhesins for host proteins. Affinity constants of staphylococcal adhesins and toxins show a direct correlation with the production of human disease (Lower *et al.*, 2011; Tkaczyk *et al.*, 2012). This problem may be complicated by the observation that human and animal proteins do not bind staphylococcal adhesins identically (Foster *et al.*, 2014). The situation is made more difficult when one considers that even preventing 99 % of staphylococci binding to an implanted device may not prevent infection because of the relatively low inoculum needed to cause infection of foreign bodies (Elek and Conen, 1957). Thus, asking monoclonal antibodies to prevent infection by competing with adhesins is a tall order.

The clinical problem is different when this approach is applied to the neutralisation of staphylococcal toxins, wherein efficacy in animal models can be directly correlated with monoclonal antibody affinity (Tkaczyk *et al*., 2012) and levels of anti-toxin antibodies in humans correlate with reduced severity of infections (Foster *et al.*, 2014). These anti-toxin antibody actions are summarised within a box in Fig. 1. In addition, antibody-staphylococcal interactions can also activate the IFN-γ pathway for clearance of *S. aureus* in mouse models (Pancari *et al.,* 2012). A model for *S. aureus* adaptive immunological responses in humans is shown (Fig. 1).

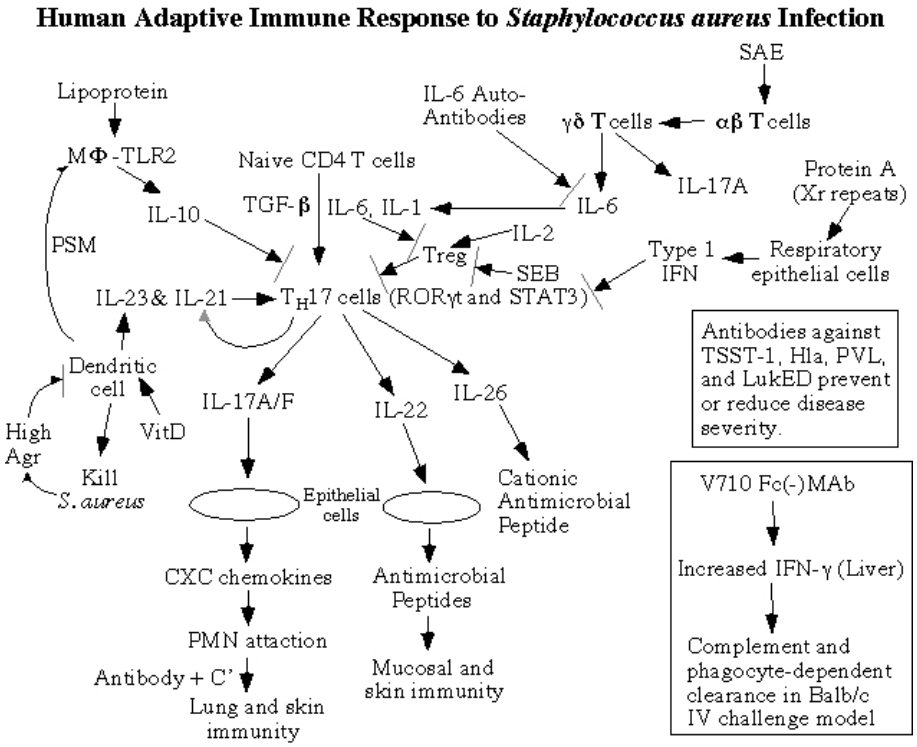

Fig. 1. A model for *S. aureus* adaptive immunological responses in humans. The exceptions are for SEB (staphylococcal enterotoxin B) wherein it interacts with regulatory T cells (Treg; $CD4^+Foxp3^+$) and V710 MAb in the liver, which is based upon murine data. For the most part, toxin responses are similar between human and animal model responses. Positive actions are indicated with arrows, whereas inhibitory responses are with blocked arrows. Abbreviations MΦ macrophage; PSM, phenol-soluble modulins; IL, interleukin; TGF-β, transforming growth factor-β; Th17, T helper type 17 cells; mAb, monoclonal antibody; V710, IsdB (iron-regulated surface determinant) antigen used in Merck Vaccine trial; C', complement; SAE, *S. aureus* enterotoxin E; RORγt, retinoid-related orphan receptor c; STAT3, signal transducer and activator of transcription 3; Hla, α-toxin; TSST-1, toxic shock syndrome toxin-1; Luk, leucocidin; PVL, Panton-Valentine leucocidin (two component toxin made up of LukS-PV and LukF-PV); IFN-γ, interferon-γ; VitD = vitamin D.

Limitations and dangers of immune activation

When considering activation of Th17 as the answer to *S. aureus* vaccine production, several cautions are needed. First, while the evidence for T cells and especially Th17 playing an important role in human immunity against *S. aureus* is gaining support (Kolata *et al.*, 2015; Misstear *et al.*, 2014; Montgomery *et al.*, 2015), Th17 immunity has not been shown to be a biomarker for protection. Second, high levels of IL-10 and low levels of IL-1 and TNF-α have been associated with worse outcomes in bacteraemia, including *S. aureus* bacteraemia (Burke *et al.*, 2013; Rose *et al.,* S (2013a) [Biomarkers in *Staphylococcus aureus* bacteraemia predicting bacteraemia duration or patient mortality. ICAAC Abstract B-1432]; Rose *et al.*, 2013b [Differential whole blood killing and IL-1 response in *Staphylococcus aureus* isolates from patients with persistent and rapidly cleared bacteraemia. ICAAC Abstract B-490]; van Dissel *et al.*, 1998). These cytokines can be related to Th17, but whether or not these cytokines will serve as biomarkers still needs to be tested in clinical vaccine trials before being accepted as biomarkers for *S. aureus* immunity. Third, the duration of Th17 immunity may be relatively short. When our standard *B. pertussis* vaccine went from a whole-cell to a subunit vaccine, the protection also shifted from being antibody-dependent to Th17-mediated immunity (Dunne *et al.*, 2010). The duration of immunity also changed from being very long-term with the whole cell vaccine to being relatively short-lived immunity with the acellular vaccine such that as children reach their early teenage years, they are developing whooping cough (Lavine *et al.*, 2012; McGirr *et al.*, 2015). There are no data about the duration of a Th17-based human immune response to *S. aureus*, but it might be equally short-lived, which would be fine for prevention of infections in ICU or implant surgeries, but it would not provide longer-term protection. Fourth, the IsdB antigen (V710 vaccine trial) did stimulate and protect by Th17, and not antibody-dependent, mechanisms (Joshi *et al.*, 2012). However, patients receiving the V710 vaccine that developed invasive *S. aureus* infections unexpectedly showed an increased incidence of multi-organ failure and death (Fowler *et al.*, 2013). Examination of cytokines in the sera from the twelve V710 recipients who died were compared to the single death amongst the, thirteen placebo recipients (McNeely *et al.*, 2014). All twelve vaccines had undetectable levels of IL-2 prior to vaccination and surgery, but only one placebo recipient with undetectable IL-2. Furthermore, nine of ten V710 recipients that had undetectable IL-17α levels preoperatively died with postoperative *S. aureus* infections. Of course, IL-2 is important for the activation of T cells, including regulatory T cells (Treg) (Smigiel *et al.*, 2014). Patients with defects in Treg have problems in maintaining immune homeostasis and develop autoimmune diseases. Other cytokines, such as Il-1 that is released during bacterial infections, can drive the conversion of Treg into Th-17 cells (Chung *et al.*, 2009; Li *et al.*, 2010). One hypothesis for the systemic inflammatory response syndrome seen in the vaccines might relate to an imbalance of Treg activity. While the mechanisms are unknown, these data suggest that the preoperative immune status and host response may predispose patients to death after priming of the immune system with vaccination. This provides a note of caution about vaccines directed at Th17 activation because Th17 has also been implicated in autoimmune diseases (Marwaha *et al.*, 2012). Such a caution is implicit in the a statement about the immune response in infection in the (Smigiel *et al.*, 2014) review: "Too much Treg cell activity can result in immunosuppression and impaired pathogen clearance, whereas too little Treg cell activity can impair effector T-cell mobilisation and avidity during infection and unleash potentially fatal inflammatory and autoimmune diseases. Identifying the cellular and molecular signals that control Treg cell homeostasis and function is essential for understanding how Treg cells influence the outcome of normal and pathological immune responses." Clearly, the model in the Fig. 1. is simply an outline of the adaptive human immune response to *S. aureus*, but a detailed and mechanistic explanation is desperately needed for the protective response in humans. Progress on the development of a safe and efficacious *S. aureus* vaccine will certainly be facilitated by investment in studies of the basic immunology of humans with *S. aureus* infections.

Conclusions

Both active and passive immunisations have been attempted, and all clinical trials have failed. These trials were based upon increased opsonophagocytic antibodies in animal models (mostly rodent) and in humans. Several more trials are ongoing, but these also are based upon opsonophagocytic antibodies, except for the rAls3p-N antigen, which focuses on Th17-mediated immunity. Previous clinical trials have used antigens (IdsB, ClfA) that stimulated the Th17 pathway and where the protection was shown to be mediated by Th17, and not antibody, have also failed to demonstrate protection (Cho *et al.*, 2010; Joshi *et al.*, 2012; Lin *et al.*, 2009; Narita *et al.*, 2010). The note of caution about active immunisation that stimulates Th17 is noted above. Similar caution applies to passive immune therapy. Anti-ClfA monoclonal antibody (tefibazumar/ Aurexis) showed a hypersensitivity reaction in one of thirty vaccine recipients (Weems *et al.*, 2006). Development of a much needed and safe staphylococcal vaccine may require a much deeper understanding of the human immune system and the development of biomarker(s) that signify protection against staphylococci.

While prevention of infection is the ultimate goal of a staphylococcal vaccine, which will be the most difficult with biomaterial infections, we may need to accept reduced morbidity *via* an anti-toxin approach for the near future. Currently, decolonisation and enhanced efforts at infection control prior to surgery appear to be the best immediate answer to reducing implant infections (Allen *et al.,* 2014; Chen *et al.*, 2013; Colling *et al.*, 2015; Huang *et al.*, 2013; Kim *et al.*, 2010; Mehta *et al.*, 2013; Schweizer *et al.,* 2013). The most studied drug for use in nasal decolonisation is mupirocin, but rapid emergence of plasmid-mediated resistance occurs where it is widely used, which reduces its value (Lee *et al.*, 2013; McDanel *et al.*,

2013; Miller *et al.*, 1996; Seah *et al.*, 2012; Vivoni *et al.*, 2005; Walker *et al.*, 2003). Combined resistance between the topical antibiotics mupirocin and retapamulin is of great concern (McNeil *et al.*, 2014). Newer agents such as XF-73 are currently being tested in phase II trials, which have a lower propensity for development of resistance (Farrell *et al.*, 2011). For example, retapamulin and mupirocin exhibit resistance in 3-5 passages, whereas XF-73 shows no resistance after 55 passages (Farrell *et al.*, 2011). The lack of XF-73 resistance is perhaps due to it extremely rapid rate of killing: > 10^5 *S. aureus* killed in 15 min at twice the minimal inhibitor concentration (Farrell *et al.*, 2010). Thus, while we await a much needed staphylococcal vaccine, screening, decolonisation and infection control are immediately available solutions to reduce the problem of prosthetic joint infections.

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Discussion with Reviewer

Reviewer I: The author's thesis that the greatest challenge in developing a protective vaccine is that "*S. aureus* is part of the normal human flora" is accepted. However, from a practical standpoint, an equal challenge is that *S. aureus* infection rates are too low for the clinical research needed to discover biomarkers of protective immunity. This challenge deserves some attention, perhaps by citing the well document ~1 % surgical site infection rates in total joint replacement and heart valve replacement.

Authors: The development of biomarkers is not dependent upon the infection rates, as one can search for biomarkers in patients with *bona fide* infections. For example, in a series of patients with *S. aureus* bacteraemia, some cytokines (high TNF-a, high IL-6, and low IL10) have been found to be markers for higher mortality (Rose W *et al.,* 2012). However, the number of subjects and the need for a large prospective trials to define these as biomarkers for severe disease are still needed. Therefore, even if only 1 % of surgical site infections were studied, presence of a cytokine or particular lymphocyte subset that correlated with cure would indicate protective immunity, and it could be found in 90 % of the 1 % of patients. Of course, I do not share the view that the infection rates are low as *S. aureus* bacteraemia is the number one cause of bacteraemia in people over 65 years old, the number one cause of children

being admitted to hospitals, and the number one cause of children having surgery. Therefore, there are plenty of cases for the study of *S. aureus* infections.

Reviewer II: Biomarkers in rodent models of *S. aureus* infections but also well-known biomarkers for *H. influenzae* and *S. pneumonia* infections in humans, have either no effect or can even have an adverse effect in *S. aureus* infections in humans. Where to your opinion do we need to look for biomarkers that will solve this problem?
Authors: We need to look to human disease responses for biomarkers. There is already human data for anti-toxin antibodies being protective against toxic shock syndrome and there are clinical trials underway examining the value of anti-alpha toxin antibodies in human disease. These antibodies are most likely to reduce disease severity. Of note, animal models and human responses are parallel, suggesting that these models will be predictive of human outcomes. Where we lack biomarkers are for disease prevention. Because animal models have performed so poorly (high levels of protection in animal models and failure to protect in human vaccine trails), one will need to look to human studies for the discovery and vetting of biomarkers.

Reviewer III: Considering the obvious challenges in developing vaccines for *S. aureus*, as outlined in the review, should we revise downwards our expectation for a successful vaccine in this context? For example, should we accept that a reduction in severity of infection be the target goal? With this in mind, would any of the current "failed" vaccines satisfy such reduced criteria?
Authors: I agree that we should revise our expectations for a successful vaccine, wherein reduction of disease severity would be the goal. Presumably, this might produce fewer ICU admissions, shorter time in ICU, shorter hospital stays, and reduced hospital costs. Clearly, these would be highly desired outcomes. To date, none of the clinical trials has been designed to examine these outcomes, but none of the clinical trials found evidence for this, except for the use of Anti-ClfA monoclonal antibodies (tefibazumab = Aurexis by Inhibitex) as passive immunisation for S. aureus bacteraemia in a phase 2 trial showed decreased relapses and complications of the infection. Unfortunately, there was no reduced mortality and hypersensitivity developed in 1 of 30 patients (Weems *et al*.). Two other trials using ClfA as an antigen have not been reported to show this result.

Editor's Note: Scientific Editor in charge of the paper: R. Geoff Richards.